ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2008, p. 2253–2255 Vol. 52, No. 6
0066-4804/08/$08.00+0 doi:10.1128/AAC.01536-07


# Interactions of DB75, a Novel Antimalarial Agent, with Other Antimalarial Drugs In Vitro▽

Anne E. Purfield, Richard R. Tidwell, and Steven R. Meshnick*

*Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina 27599-7435*

Received 28 November 2007/Returned for modification 20 January 2008/Accepted 11 March 2008

**Pafuramidine is a novel orally active antimalarial. To identify a combination partner, we measured the in vitro antimalarial activities of the active metabolite, DB75, with amodiaquine, artemisinin, atovaquone, azithromycin, chloroquine, clindamycin, mefloquine, piperaquine, pyronaridine, tafenoquine, and tetracycline. None of the drugs tested demonstrated antagonistic or synergistic activity in combination with pafuramidine.**

Pafuramidine (DB289), 2,5-bis-(4-amidinophenyl)furan-bis-*O*-methylamidoxime, is a promising new orally active antiparasitic compound (9). A recent clinical study found that pafuramidine (200 mg/kg of body weight/day for 5 days) was effective for 96% (22 of 23) of *Plasmodium falciparum*-infected patients (9). In vivo, pafuramidine is metabolized into the active metabolite 2,5-bis-(4-amidinophenyl)furan (DB75) (10, 11). Previous studies have shown that DB75 is effective against *P. falciparum*, *Plasmodium vivax*, *Pneumocystis jirovecii*, *Trypanosoma brucei*, and *Leishmania* spp. in vitro and in animal models (2, 7).

Currently, the WHO recommends that all new antimalarial drugs be used in combination with a second antimalarial drug to prevent the development of resistance to monotherapy. The two partnered drugs cannot be antagonistic and should ideally be synergistic (8). The purpose of this study was to characterize the interactions of the active metabolite of pafuramidine, DB75, with potential antimalarial partner drugs.

To measure in vitro drug sensitivity, we used 42-h microdilution checkerboard growth assays and cultured *P. falciparum* 3D7 parasites as previously described (4). Serial dilutions of DB75 were tested in combination with serial dilutions of each partner drug: amodiaquine, artemisinin, atovaquone, azithromycin, chloroquine, clindamycin, mefloquine, piperaquine, pyronaridine, tafenoquine, and tetracycline (3). Drug concentrations ranged from 5- to 10-fold above and below the predetermined 50% inhibitory concentration ($IC_{50}$). For each partner drug, the assay was repeated two or three times. The atovaquone-proguanil combination was used to validate the method and to determine the threshold of the sum of fractional inhibitory concentrations (FIC) for synergism.

Additionally, 66-h and 96-h exposure periods were used for select drugs (tetracycline, clindamycin, azithromycin, and tafenoquine) with long onsets in vitro. To measure growth in 66 h, the methods described above were followed except that a 0.4% parasite population was used and [$^3$H]hypoxanthine was added for the final 18 h of incubation. Ring cultures synchronized with 0.3% sorbitol were used for the 96-h assay, and [$^3$H]hypoxanthine was added for the final 48 h.

Results were expressed as FIC and as the mean sums of the FIC. DB75–partner drug interactions were defined as either indifferent (linear points on isobolograms; sum of FIC = 1), synergistic (concave curves on isobolograms; sum of FIC = ≤0.63) (atovaquone and proguanil), or antagonistic (convex curves on isobolograms; sum of FIC = >2.0) (1, 3).

To find a potential partner for DB75, the compound was tested in combination with 11 current and investigational antimalarials. DB75 manifested indifferent relationships with 10 of the drugs tested in the 42-h assay by both the FIC and sum-of-FIC methods (Fig. 1; Table 1). As a positive control, atovaquone and proguanil showed clear synergy (sum of FIC = 0.63).

Three of these drugs (tetracycline, tafenoquine, and azithromcycin) also showed indifference even with extended incubations (Fig. 1F, D, and B, respectively; Table 1).

The clindamycin-DB75 combination was indifferent at 96 h (Fig. 1L; Table 1). As previously reported, clindamycin alone exhibited a biphasic dose response by the 42- and 66-h assays (data not shown) (5). Complete inhibition was not achieved

TABLE 1. Results for antimalarial agents in combination with DB75

| Partner drug | FIC (sum of FIC)[a] from the following assay: | | |
|---|---|---|---|
| | 42-h | 66-h | 96-h |
| Clindamycin | N/A (N/A) | N/A (N/A) | I (1.09 ± 0.16)[b] |
| Tetracycline | I (0.88 ± 0.08) | I (1.04 ± 0.19) | |
| Atovaquone | I (0.94 ± 0.14) | | |
| Tafenoquine | I (1.00 ± 0.06) | I (1.05 ± 0.06) | |
| Mefloquine | I (1.08 ± 0.14) | | |
| Amodiaquine | I (1.09 ± 0.03) | | |
| Artemisinin | I (1.19 ± 0.15) | | |
| Piperaquine | I (1.23 ± 0.04) | | |
| Azithromycin | I (1.20 ± 0.18) | I (1.29 ± 0.13) | |
| Pyronaridine | I (1.20 ± 0.02) | | |
| Chloroquine | I (1.31 ± 0.12) | | |
| Atovaquone and proguanil | S (0.63) | | |

[a] FIC sums are reported as means ± standard deviations from two to three assays. S, synergistic; I, indifferent; N/A, not applicable. Mean DB75 $IC_{50}$s ± standard deviations are 128 ± 51 nM at 42 h, 139 ± 43 nM at 66 h, and 3.7 ± 0.76 nM at 96 h.

[b] Synchronized rings were used for the 96 h assay.

* Corresponding author. Mailing address: Department of Epidemiology, CB 7435, University of North Carolina, Chapel Hill, NC 27599-7435. Phone: (919) 966-7414. Fax: (919) 966-2089. E-mail: meshnick@unc.edu.

▽ Published ahead of print on 24 March 2008.

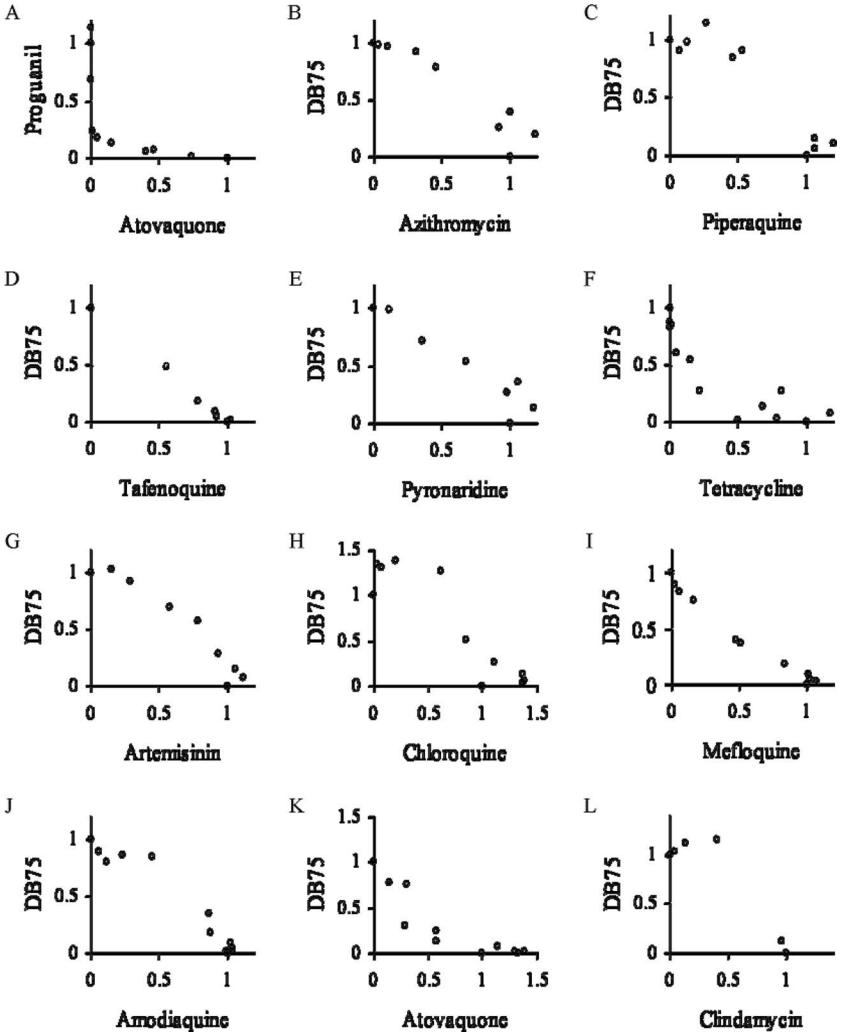


FIG. 1. Isobolograms of DB75–partner drug interactions. (A, C, E, and G to K) Growth was measured by using 42-h [$^3$H]hypoxanthine growth assays for all drugs tested in combination with DB75. (A) Atovaquone and proguanil were used in combination to show true synergy at 42 h. (B, D, and F) Azithromycin (B), tafenoquine (D), and tetracycline (F) were also tested in combination with DB75 for 66 h. (L) Clindamycin was tested at 96 h. A concave isobologram (A) indicates synergy; a convex one (none shown) indicates antagonism; and points along a straight line (B to L) indicate indifference.

with clindamycin at these times, even with hemolytic concentrations greater than 100 μM; therefore, no $IC_{50}$ could be extrapolated for the 42- or 66-h assay. However, at 96 h, a sigmoidal dose response was evident for clindamycin alone; therefore, an FIC could be determined.

In summary, the lack of interaction of DB75 with either chloroquine or atovaquone suggests that DB75 does not interact with hemozoin or mitochondria in the parasite, as has been previously suggested (6). These data also suggest that pafuramidine could be successfully partnered with any of the 11 antimalarials tested here. Follow-up studies in animal models are needed.

Funding for this work was provided by Medicines for Malaria Venture and the Bill and Melinda Gates Foundation.

We acknowledge Carla Hand, Jesse Kwiek, Jeff Frelinger, Carrie Barnes, David Klapper, Stephanie Wallace, and Jaina Patel for technical assistance.

## REFERENCES

1. **Berenbaum, M. C.** 1978. A method for testing for synergy with any number of agents. J. Infect. Dis. **137:**122–130.
2. **Brendle, J. J., A. Outlaw, A. Kumar, D. W. Boykin, D. A. Patrick, R. R. Tidwell, and K. A. Werbovetz.** 2002. Antileishmanial activities of several classes of aromatic dications. Antimicrob. Agents Chemother. **46:**797–807.
3. **Canfield, C. J., M. Pudney, and W. E. Gutteridge.** 1995. Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum* in vitro. Exp. Parasitol. **80:**373–381.
4. **Desjardins, R. E., C. J. Canfield, J. D. Haynes, and J. D. Chulay.** 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. **16:**710–718.
5. **Seaberg, L. S., A. R. Parquette, I. Y. Gluzman, G. W. Phillips, Jr., T. F. Brodasky, and D. J. Krogstad.** 1984. Clindamycin activity against chloroquine-resistant *Plasmodium falciparum*. J. Infect. Dis. **150:**904–911.
6. **Stead, A. M., P. G. Bray, I. G. Edwards, H. P. DeKoning, B. C. Elford, P. A. Stocks, and S. A. Ward.** 2001. Diamidine compounds: selective uptake and targeting in *Plasmodium falciparum*. Mol. Pharmacol. **59:**1298–1306.
7. **Tidwell, R. R., S. K. Jones, J. D. Geratz, K. A. Ohemeng, C. A. Bell, B. J. Berger, and J. E. Hall.** 1990. Development of pentamidine analogues as new agents for the treatment of *Pneumocystis carinii* pneumonia. Ann. N. Y. Acad. Sci. **616:**421–441.

8. **WHO.** 2001. Antimalarial drug combination therapy. World Health Organization, Geneva, Switzerland.
9. **Yeramian, P., S. R. Meshnick, S. Krudsood, K. Chalermrut, U. Silachamroon, N. Tangpukdee, J. Allen, R. Brun, J. J. Kwiek, R. Tidwell, and S. Looareesuwan.** 2005. Efficacy of DB289 in Thai patients with *Plasmodium vivax* or acute, uncomplicated *Plasmodium falciparum* infections. J. Infect. Dis. **192:**319–322.
10. **Zhou, L., K. Lee, D. R. Thakker, D. W. Boykin, R. R. Tidwell, and J. E. Hall.** 2002. Enhanced permeability of the antimicrobial agent 2,5-bis(4-amidinophenyl)furan across Caco-2 cell monolayers via its methylamidoxime prodrug. Pharm. Res. **19:**1689–1695.
11. **Zhou, L., D. R. Thakker, R. D. Voyksner, M. Anbazhagan, D. W. Boykin, J. E. Hall, and R. R. Tidwell.** 2004. Metabolites of an orally active antimicrobial prodrug, 2,5-bis(4-amidinophenyl)furan-bis-*O*-methylamidoxime, identified by liquid chromatography/tandem mass spectrometry. J. Mass Spectrom. **39:**351–360.